C3 Production of Cultured Human Epidermal Keratinocytes is Enhanced by IFNγ and TNFα through Different Pathways

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We investigated the regulation of C3 production by human cultured epidermal keratinocytes by enzyme-linked immunosorbent assay. The results showed that IFNγ and TNFα enhanced the synthesis of C3 by epidermal keratinocytes in a concentration-dependent manner. Moreover, a protein kinase C (PKC) inhibitor blocked C3 production, whereas PMA enhanced it. There was a synergistic effect between IFNγ and TNFα. In experiments to investigate the role of protein tyrosine kinase (PTK) in C3 production, we found that treatment with herbimycin A, a specific inhibitor for the c-Src-related PTK, caused significant enhancement of the C3 production induced by IFNγ or TNFα, suggesting that c-Src-type PTK(s) provides a negative signal to C3 production. Each competitive inhibitor of PTK, genistein or tyrphostin, substantially increased the C3 production by IFNγ at lower concentrations, although each agent had little effect on TNFα-associated production of C3 at the same concentrations. The data show that pro-inflammatory cytokines IFNγ and TNFα synergistically augment C3 production by epidermal keratinocytes by different pathways. ***Key words: C3/human keratinocyte/IFNγ/TNFα. J Invest Dermatol 108:62–67, 1997***

In general, liver hepatocytes are regarded as the major source of most complement components (Alper *et al*, 1969), but some of these components are also synthesized at extrahepatic sites by other cells such as monocytes/macrophages (Sackstein and Colten, 1984; Hamilton *et al*, 1987; Kulics *et al*, 1990), polymorphonuclear leukocytes (Botto *et al*, 1992) fibroblasts (Katz *et al*, 1989; Katz and Strunk, 1989), endothelial cells (Brooimans *et al*, 1990; Lappin *et al*, 1992), and epithelial cells (Strunk *et al*, 1988; Brooimans *et al*, 1991; Andoh *et al*, 1993). Human epidermal keratinocytes have also been reported as candidates for local biosynthetic sites for C3 (Basset-Seguin *et al*, 1990) and factor B (Yancey *et al*, 1992). Because several humoral factors, including cytokines, regulate complement synthesis in the liver (Permutter *et al*, 1986a: Permutter *et al*, 1986b; Ramadori *et al*, 1988) or other tissues, we assume that these factors might also participate in the regulation of complement synthesis in the skin.

The regulatory mechanisms involved in systemic or local complement synthesis have been only partially elucidated. For example, pro-inflammatory cytokines, such as interleukin-1 (IL-1), tumor necrosis factor-α (TNFα), or interleukin-6 (IL-6), were found to selectively increase the synthesis of C3 or factor B in hepatocytes (Perlmutter *et al*, 1986a: Perlmutter *et al*, 1986b; Ramadori *et al*, 1988), human fibroblasts (Katz *et al*, 1989; Katz and Strunk, 1989), and an intestinal epithelial cell line (Andoh *et al*, 1993). In another study, interferon-γ (IFNγ) was demonstrated to enhance the synthesis of C2, C4, and factor B (Kulics *et al*, 1990; Lappin *et al*, 1992), although it did not affect the C3 production in human monocytes, fibroblasts, a hepatoma cell line (Lappin *et al*, 1992), or in umbilical vein endothelial cells (Brooimans *et al*, 1990; Lappin *et al*, 1992). In the current study we designed a series of experiments to investigate the cytokine regulation of C3 production by human epidermal keratinocytes and to further analyze the signal transduction pathways involved in the C3 production augmented by these cytokines.

Manuscript received March 14, 1996; revised May 29, 1996; accepted for publication September 30, 1996.

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Abbreviations: PTK, protein tyrosine kinase; KGM, keratinocyte growth medium.

MATERIALS AND METHODS

Chemicals and Reagents Human cytokines were purchased or provided as follows: IL-1α, IL-1β, IL-4, IL-6, IL-7, IL-8, TNFα (Genzyme, Cambridge, MA), IL-10, transforming growth factor-β1 (TGFβ1), granulocyte-macrophage colony stimulating factor (R & D Systems, Minneapolis, MN), IL-3, IFNβ, IFNγ (GIBCO BRL, Gaithersburg, MD), IL-5 (Wako, Tokyo, Japan), TGFα (Boehringer Mannheim GmbH, Mannheim, Germany), and IFNα2a (a gift from Takeda Pharmaceutical Co., Osaka, Japan). All the cytokines, prepared at higher concentrations in 0.1% bovine serum albumin-containing phosphate-buffered saline, were stocked at −70°C and diluted in the complete medium just before use for tissue cultures. PTK inhibitors, herbimycin A, genistein, and tyrphostin, were obtained from GIBCO BRL. A protein kinase C (PKC)-specific inhibitor, Ro31-8220, was a gift from Dr. Bradshaw of Roche Research Center (London, UK). Phorbol myristate acetate (PMA), forskolin, and sodium ortho-vanadate were purchased from Sigma (St. Louis, MO).

Culture of Epidermal Keratinocytes Human epidermal keratinocytes derived from neonatal foreskin or adult breast were obtained from Clonetics Corporation (San Diego, CA) and expanded in modified serum-free MCBD 153 media (*pH* 7.4, *osmolarity* 340 ± 4 mOsm/kg; 0.15 mM Ca^{2+}), containing growth factors including bovine pituitary extract, or keratinocyte growth medium (KGM; Clonetics, San Diego, CA). The cells in the

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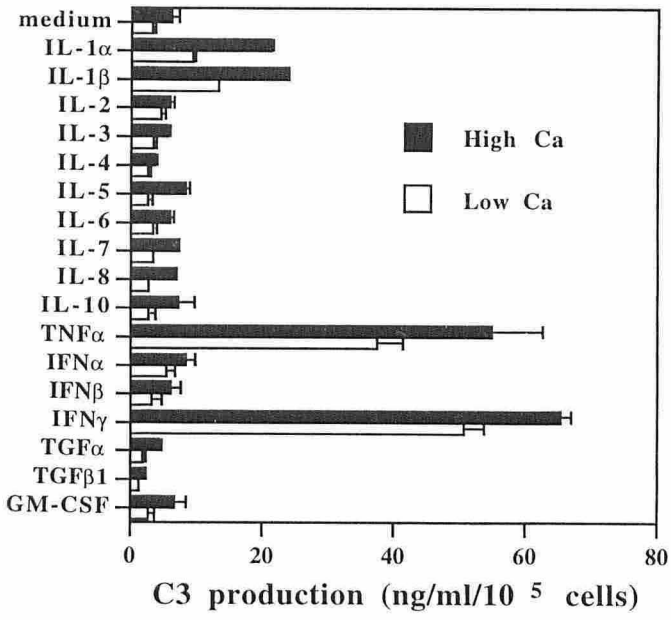

Figure 1. IFNγ and TNFα stimulate C3 production by cultured human epidermal keratinocytes, and the state of keratinocyte differentiation influences C3 production. Human epidermal keratinocytes were expanded and treated in KGM containing low Ca^{2+} (0.15 mM) for 48 h with each cytokine: IL-1α (100 U per ml), IL-1β (100 U per ml), IL-2 (200 U per ml), IL-3 (10 ng per ml), IL-4 (200 U per ml), IL-5 (10 ng per ml), IL-6 (200 U per ml), IL-7 (10 ng per ml), IL-8 (10 ng per ml), IL-10 (50 ng per ml), TNFα (100 ng per ml), IFNα2a (200 U per ml), IFNβ (200 U per ml), IFNγ (200 U per ml), TGFα (10 ng per ml), TGFβ1 (10 ng per ml), or GM-CSF (10 ng per ml). To induce keratinocyte differentiation, keratinocytes were incubated in KGM containing high Ca^{2+} (1.2 mM) for 24 h. They were further cultured in the presence or absence of each cytokine for 48 h. The supernatants were assayed for C3 by ELISA. C3 production was expressed per 10^5 viable keratinocytes (nanograms per ml/10^5 cells) assessed by use of trypan blue dye. Data are mean ± SD.

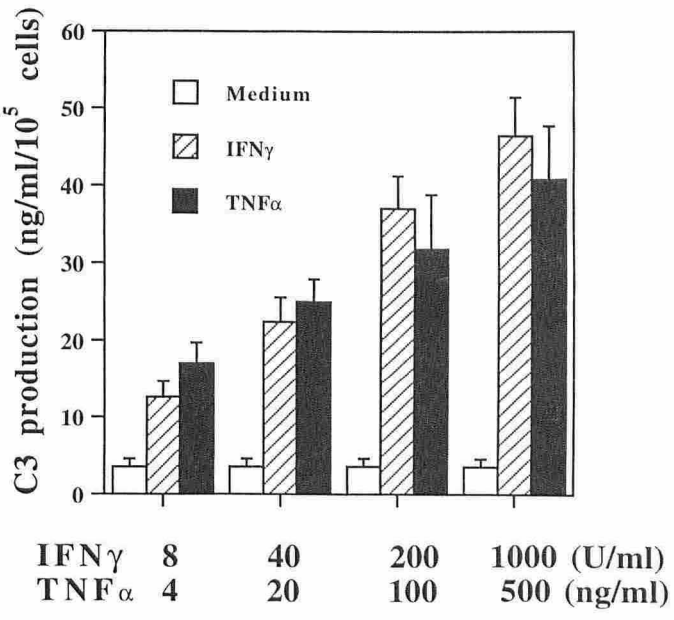

Figure 2. IFNγ and TNFα increase C3 production by epidermal keratinocytes in a concentration-dependent fashion. Epidermal keratinocytes cultured in KGM containing low Ca^{2+} (0.15 mM) were stimulated with increasing amounts of each cytokine for 48 h: IFNγ (200 U per ml), TNFα (100 ng per ml). The supernatants were assayed for C3 by ELISA. C3 production was expressed per 10^5 viable keratinocytes assessed (nanograms per ml/10^5 cells) by use of trypan blue dye. Data are mean ± SD.

third passage were cultured in KGM containing low Ca^{2+} (0.15 mM) in 48- or 24-well flat-bottomed microtiter plates (Coster, Cambridge, MA) to attain 70–80% confluency. In the first experiment **(Fig 1)** some of the keratinocytes were cultured in high concentrations of Ca^{2+} (1.2 mM) for 24 h to induce keratinocyte differentiation and then treated with each cytokine. In the remaining experiments they were cultured in KGM containing low concentrations of Ca^{2+} (0.15 mM). In other experiments these keratinocytes were pretreated with protein kinase antagonists or agonists for the indicated periods of times, and they were then treated with each cytokine.

Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) for the Quantification of C3 The C3 concentrations in samples were quantified by sandwich ELISA. The wells of 96-well flat-bottomed microtiter plates (Dynatech Laboratories, Chantilly, VA) were coated with polyclonal goat anti-human C3 (IgG fraction; Cappel, West Chester, PA) in a 50 mM carbonate/bicarbonate buffer (pH 9.6) for 16 h at 4°C. After washing and blocking of nonspecific protein binding, the samples were incubated in the wells for 2 h at 37°C. Next, the wells were thoroughly washed and incubated with peroxidase-labeled goat anti-human C3 (IgG fraction; Cappel) for 2 h at 37°C. Color development of all samples was performed using 1,2-o-phenylenediamine dihydrochloride (Sigma, St. Louis, MO) as a substrate. Phosphate-buffered saline containing 0.05% Tween 20 was used for washing. The amount of the reacted substrate was determined by OD_{492}. Highly purified human C3 (Sigma, St. Louis, MO) was used as a standard. The lower limit of this ELISA was 10 ng of C3 per ml, and no cross-reactivity was detected in fetal bovine serum. After the supernatants were removed at the end of each cytokine treatment, cultured keratinocytes were treated with trypsin-ethylenediamine tetraacetic acid solution and the number of detached cells were counted. C3 production was expressed per 10^5 viable keratinocytes assessed by use of trypan blue dye. All pharmacologic agents had little effect on keratinocyte viability at concentrations used in this experiment. Each experiment was done in triplicate and repeated at least three times, and means and standard deviations of representative results are shown in the figures. Experimental data were analyzed using Student's t test.

RESULTS

IFNγ and TNFα Augment C3 Synthesis by Human Cultured Keratinocytes As reported by another laboratory (Basset-Seguin *et al*, 1990), cultured keratinocytes had the ability to produce C3 constitutively. To determine the effects of various cytokines produced by human epidermal keratinocytes or inflammatory cells on the synthesis of C3, the keratinocytes were cultured in KGM containing a set of growth factors including epidermal growth factor and low Ca^{2+} (0.15 mM) for 48 h in the presence or absence of cytokines. Our results show that keratinocytes produced C3 vigorously in the presence of TNFα or IFNγ **(Fig 1)**.

We also examined the influence of the state of keratinocyte differentiation. To induce keratinocyte differentiation, keratinocytes were incubated in KGM containing high Ca^{2+} (1.2 mM) for 24 h. They were further cultured in the presence or absence of each cytokine, and data obtained were compared with those obtained with KGM containing low Ca^{2+} (0.15 mM). The result **(Fig 1)** showed that they produced higher amounts of C3 ($p < 0.05$) in high Ca^{2+} conditions, suggesting that the state of keratinocyte differentiation influences C3 production. In later experiments the keratinocytes were cultured in KGM containing low Ca^{2+} (0.15 mM) in the presence or absence of cytokines.

The enhancement by TNFα or IFNγ was found to be concentration dependent **(Fig 2)**. The result of time course study showed that small amounts of C3 were detected at 24 h in response to IFNγ (200 U per ml) or TNFα (100 ng per ml), and the production was increased considerably in the next 24 h **(Fig 3)**. IL-1α and IL-1β had minute effects only at high concentrations. Other cytokines showed no influence on the C3 production by keratinocytes at even higher concentrations than shown in **Fig 1** (data not shown).

The Cytokine Regulation of C3 Production by Keratinocytes Is Dependent on PKC Activity An increment of the Ca^{2+} concentration in the culture medium induces the differentiation of keratinocytes through PKC activation (Matsui *et al*, 1992).

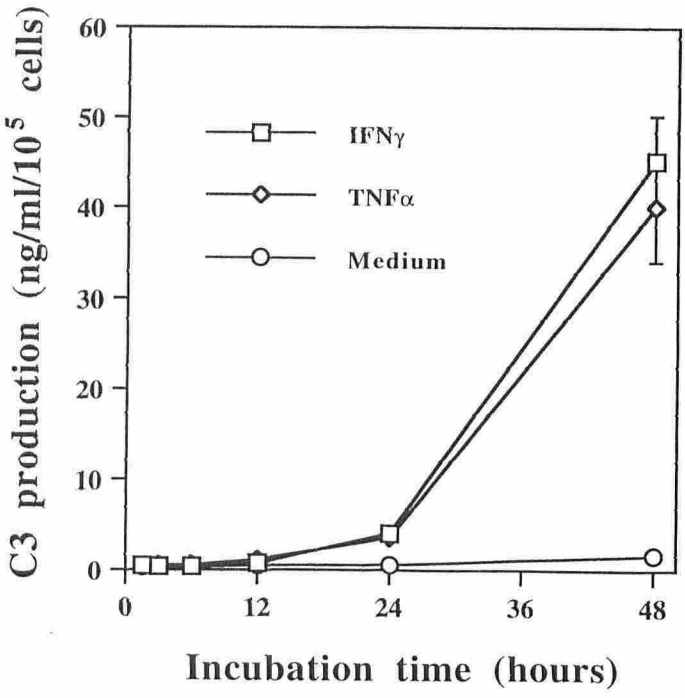

Figure 3. IFNγ or TNFα enhances C3 production by epidermal keratinocytes in a time-dependent manner. Epidermal keratinocytes cultured in KGM containing low Ca^{2+} (0.15 mM) were stimulated with TNFα (100 ng per ml) or IFNγ (200 U per ml) for 48 h. The supernatants were obtained at indicated times as shown and assayed for C3. C3 production was expressed per 10^5 viable keratinocytes (nanograms per ml/10^5 cells). Data are mean ± SD.

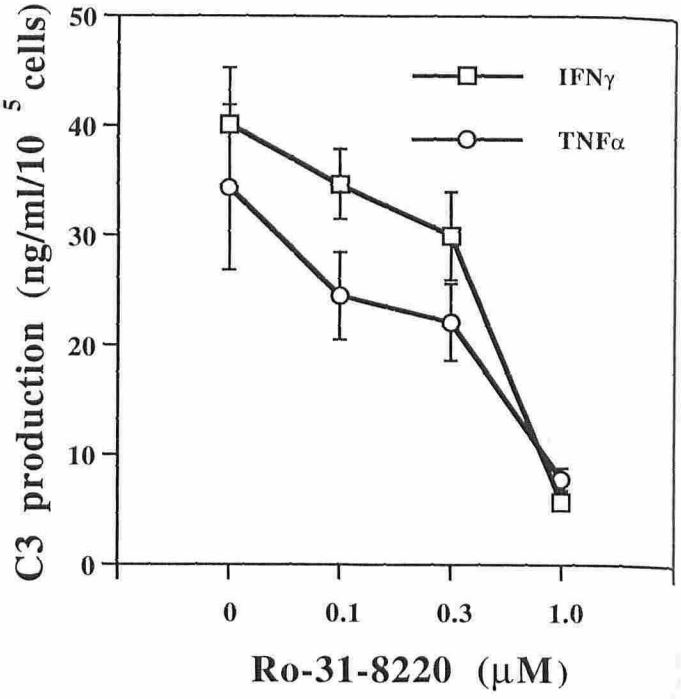

Figure 4. A selective PKC inhibitor, Ro31-8220 blocks C3 production by epidermal keratinocytes. Cultured keratinocytes in KGM containing low Ca^{2+} (0.15 mM) were pretreated with Ro31-8220 for 1 h and subsequently cultured for 48 h in the presence of IFNγ (200 U per ml) or TNFα (100 ng per ml). C3 production was assessed by ELISA and expressed per 10^5 viable keratinocytes (nanograms per ml/10^5 cells). Data are mean ± SD.

In the first set of experiments to investigate the association of the PKC-dependent pathway with the C3 production, we examined the effect of a highly selective PKC inhibitor, Ro31-8220, on C3 production. Pre-treatment with this compound for 1 h inhibited the C3 production induced by IFNγ or TNFα in a concentration-dependent manner **(Fig 4)**, although this inhibitor had little effect on keratinocyte viability at concentrations used in this experiment (data not shown). We also added a PKC agonist, PMA, and an adenylate cyclase activator, forskolin (0.1 μM), as a cAMP-dependent kinase agonist to the cultured keratinocytes. PMA (0.1 μM) treatment for 15 min increased the C3 production enhanced by IFNγ or TNFα, whereas forskolin treatment for 1 h did not affect the C3 production **(Fig 5)**.

IFNγ and TNFα Augment C3 Production by Keratinocytes Through the Activation of Different Sets of PTKs To further characterize the signal transductional pathways to induce C3 production by IFNγ and TNFα, we examined whether PTKs are associated with the C3 production induced by these cytokines using three different kinds of PTK inhibitors and a protein tyrosine phosphatase inhibitor, sodium vanadate, utilized as a PTK agonist. Herbimycin A, a specific degradative agent for the c-Src-related PTK (Uehara *et al*, 1989), genistein, a competitive inhibitor for the ATP-binding site of PTK (Akiyama *et al*, 1987), and tyrphostin, a competitive inhibitor for the substrate binding site of PTK (Gazit *et al*, 1989), were tested for their inhibitory activity on the C3 production by keratinocytes.

Herbimycin A treatment of keratinocytes for 16 h enhanced the C3 production in a concentration-dependent fashion **(Fig 6*a*)**, suggesting that a c-Src family of PTK provides a negative signal to C3 production. Interestingly, pre-incubation of epidermal keratinocytes with genistein for 1 h or with tyrphostin for 16 h showed different effects on the C3 production induced by IFNγ or TNFα **(Fig 6*b,c*)**. High concentrations of genistein and tyrphostin exerted a slightly inhibitory effect on the increase in C3 production by IFNγ or TNFα. On the other hand, at lower concentrations both inhibitors enhanced the C3 production induced by IFNγ, but not that by TNFα **(Fig 6*b,c*)**. Treatment of epidermal keratinocytes with a protein tyrosine phosphatase inhibitor, sodium vanadate, for 6 h enhanced the C3 production in a concentration-dependent

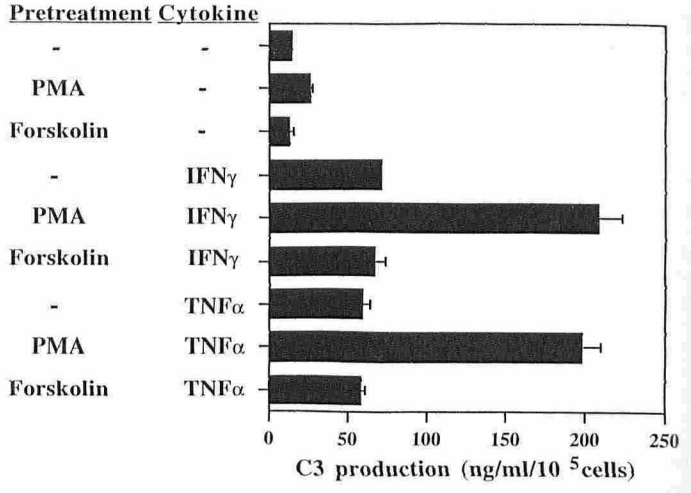

Figure 5. PMA enhances the C3 production by IFNγ or TNFα. Epidermal keratinocytes cultured in KGM containing low Ca^{2+} (0.15 mM) were pretreated with PMA for 15 min or forskolin for 1 h and further cultured for 48 h in the presence or absence of IFNγ (200 U per ml) or TNFα (100 ng per ml). The supernatants were assayed for C3 by ELISA. C3 production was expressed per 10^5 viable keratinocytes (nanograms per ml/10^5 cells). Data are mean ± SD.

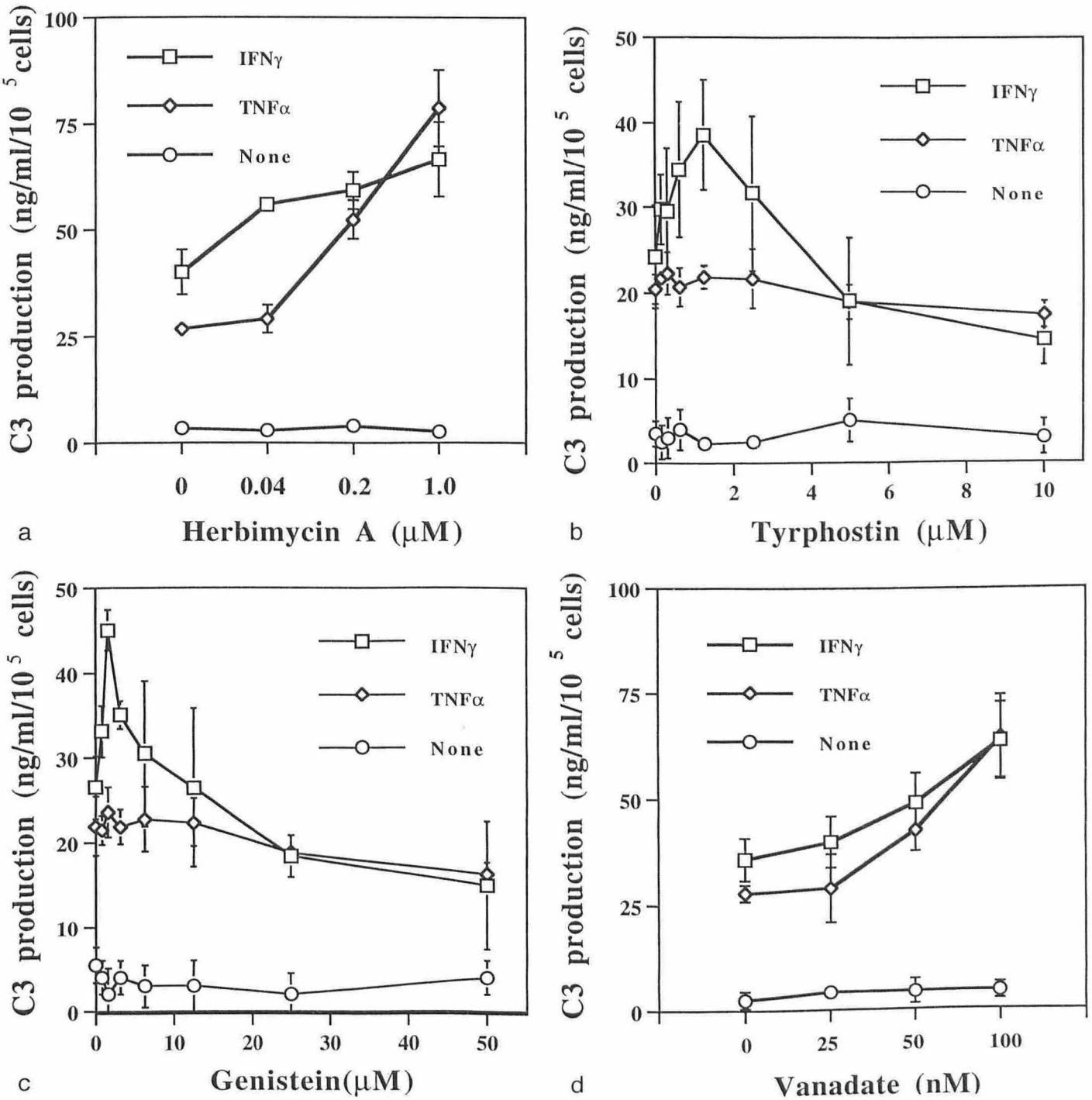

Figure 6. IFNγ and TNFα augment C3 production by different PTK-associated signal transduction pathway(s). Human epidermal keratinocytes were expanded and pretreated with either PTK antagonist or agonist for the following times in KGM containing low Ca^{2+} (0.15 mM). PTK inhibitors: herbimycin A *(a)* and tyrphostin *(b)* for 16 h and genistein *(c)* for 1 h; a protein tyrosine phosphatase inhibitor, sodium vanadate, was used as a PTK agonist *(d)* for 6 h. They were further cultured in the presence of IFNγ (200 U per ml) or TNFα (100 ng per ml) for 48 h. The supernatants were assayed for C3 by ELISA. C3 production was expressed per 10^5 viable keratinocytes (nanograms per ml/10^5 cells). Data are mean ± SD.

fashion **(Fig 6*d*)**, indicating that the modulation of C3 production by IFNγ or TNFα is associated with PTK activity. From this and other studies, we assume that different sets of PTKs with different affinities against these PTK inhibitors are involved in the C3 production induced by IFNγ. All antagonists and agonist had little effect on keratinocyte viability and constitutive production of C3 **(Figs 6*a-d*)** at concentrations used in this experiment.

IFNγ and TNFα Synergistically Enhance C3 Production by Keratinocytes The results of the previous experiments suggested that IFNγ and TNFα enhanced C3 production by keratinocytes through different pathways. In the next experiment we investigated whether IFNγ and TNFα exert a synergistic effect on the C3 production by keratinocytes. Keratinocytes were preincubated at first with only a small amount (8 U per ml for IFNγ or 4 ng per ml for TNFα) of either one of these cytokines for 1 h and further incubated with different concentrations of the other or the same cytokine for the next 48 h, as shown in **Fig 7**. Compared with the results of the C3 production induced by a single cytokine, either

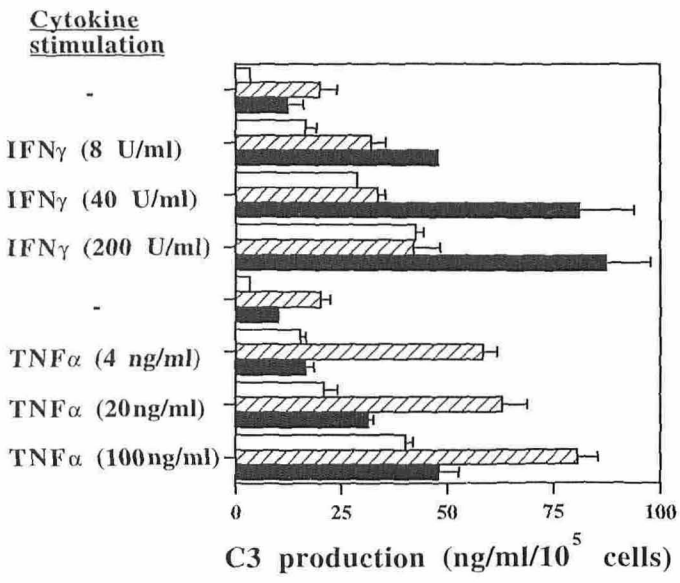

Figure 7. IFNγ and TNFα have a synergistic effect on C3 production by epidermal keratinocytes. Epidermal keratinocytes were expanded in KGM containing low Ca^{2+} (0.15 mM). They were pre-treated with a small amount of either cytokine (8 U per ml for IFNγ or 4 ng per ml for TNFα) for 1 h and further incubated with increasing amounts of the other or the same cytokine for the next 48 h. The supernatants were assayed for C3 by ELISA. C3 production was expressed per 10^5 viable keratinocytes (nanograms per ml/10^5 cells). Data are mean ± SD. Pretreatment: □, not treated; ▨, IFNγ (8 U per ml); ■, TNFα (4 ng per ml).

treatment combined with a different cytokine caused a marked enhancement, almost reaching twice that attained with a single cytokine treatment **(Fig 7)**. On the other hand, combination treatment with the same cytokine showed only an additive effect **(Fig 7)**. These results also support the observation that the C3 production is not induced through the same signal transduction pathway(s).

DISCUSSION

There is accumulating evidence that epidermal keratinocytes can initiate and propagate immunologic and inflammatory reactions through the action of cytokines they secrete. In the current study, we demonstrated that pro-inflammatory cytokines IFNγ or TNFα enhance the production of an important complement component, C3, by epidermal keratinocytes. In this experiment we have evaluated C3 production by an ELISA method. There are published data (Yancey *et al*, 1992) showing that C3 produced by epidermal keratinocytes corresponds structurally and functionally to C3 found in plasma studied by different methods.

IFNγ can irreversibly inhibit keratinocyte proliferation (Saunders and Jetten, 1994). The inhibition of keratinocyte growth by IFNγ is accompanied by a remarkable reduction in the expression of several growth control genes. In addition to IFNγ, there are several factors that influence keratinocyte growth. Among them, epidermal growth factor, TGFα, and IL-6 are known to stimulate the proliferation of epidermal keratinocytes. Conversely, TGFβ1 induces reversible growth arrest in epidermal keratinocytes (George *et al*, 1990). Our result showed that epidermal growth factor, TGFα, IL-6, and TGFβ1 were not found to influence C3 production, indicating that the signal transductional pathway for C3 production is not necessarily associated with the signal for cell growth.

Several factors, including elevation of Ca^{2+}, PKC, retinoids, and IFNγ, have been shown to influence epidermal differentiation. Because phorbol ester-mediated activation of PKC induces squamous differentiation in epidermal keratinocytes (Yuspa *et al*, 1982; Reiners and Slaga, 1983), the induction of squamous differentiation by IFNγ could be mediated by the activation of PKC. In our experiments we demonstrated that C3 production was associated with keratinocyte differentiation because pre-incubation of keratinocytes in a high Ca^{2+} condition or addition of a PKC agonist enhanced C3 production, whereas a PKC antagonist inhibited the production effectively, indicating that the C3 production induced by IFNγ is dependent on PKC activation.

TNFα is known to be produced by epidermal keratinocytes (Kock *et al*, 1990) as well as by macrophages and lymphocytes. The binding of TNFα to its receptors triggers the activation of several second messengers through stimulation of PKC (Brenner *et al*, 1989), sphingomyelinase (Yang *et al*, 1993), and phospholipase A2 (Hayakawa *et al*, 1993). Cell exposure to TNFα also results in NFκB activation (Lenardo and Baltimore, 1989) and stimulation of AP-1 activity (Brenner *et al*, 1989; Westwick *et al*, 1994). We found in this study that C3 production by TNFα was inhibited by a PKC inhibitor, whereas production was enhanced significantly by a PKC agonist, PMA, supporting the idea that the C3 production induced by TNFα is dependent on the activation of PKC.

We showed that IL-1α and IL-1β had minute effects on C3 production at their high concentrations. The effects were variable between different skin donors. Although we could not obtain conclusive data in the current study, the age of skin donors and culture conditions may influence the C3 production. It is also known that IL-1 production by keratinocytes and its function are complex and are regulated, as shown by Kupper (reviewed in Kupper, 1990). Thus, we assume that several factors might modulate C3 production through the regulation of the expression of IL-1 receptor on keratinocytes or the production of IL-1 receptor antagonist (Bigler *et al*, 1992).

Phosphorylation of proteins on tyrosine residues is a key biochemical reaction that mediates numerous cellular signals, including the control of the cell cycle and cell differentiation (Hunter and Cooper, 1985). There is a report describing the inhibitory effect of PTK inhibitors, tyrphostins, on the proliferation of cultured normal human keratinocytes (Dvir *et al*, 1991). A Ca^{2+}-sensitive PTK causes keratinocytes to differentiate via subsequent PKC activation (Filvaroff *et al*, 1994). It is also known that two different PTKs, c-Src, and c-Yes, which is a member of c-Src-type PTK, are associated with keratinocyte differentiation; i.e., in proliferating keratinocytes, c-Yes kinase activity is sustained and the signal of keratinocyte differentiation inactivates its activity. On the other hand, the differentiation signal results in the enhancement of c-Src activity (Zhao *et al*, 1993), although the precise molecular mechanism is still not known. The results of our study show that a degradation of c-Src-type PTKs by herbimycin A cause a significant enhancement of C3 production by keratinocytes induced by IFNγ or TNFα, suggesting that c-Src-type PTK(s), possibly c-Src, provides a negative signal to the C3 production.

Although we demonstrated in this study that PTK activation plays a pivotal role in the IFNγ- or TNFα-induced C3 production, experiments conducted with the PTK inhibitors, genistein, and tyrphostin, showed quite different results. Both inhibitors slightly reduced the TNFα and IFNγ-associated production of C3 at higher concentrations. Interestingly, the augmentation of C3 production by IFNγ was increased rather vigorously at lower concentrations. We also demonstrated that IFNγ and TNFα exerted a synergistic effect on C3 production by keratinocytes, supporting the idea that different PTKs are involved in the pathway for C3 production augmented by IFNγ and TNFα.

The epidermis, the outermost layer of the skin, is not merely a barrier, but actively exerts a pro-inflammatory function through secretion of various modulators, including not only cytokines and peptide factors but also complement components. We demonstrated here that the pro-inflammatory cytokines, IFNγ and TNFα, augment the C3 production by epidermal keratinocytes synergistically. We think that, in pathologic conditions, where levels of IFNγ and TNFα are elevated in the skin, complement activation by invading microorganisms or stratum corneum (Terui *et al*, 1989;

Terui *et al*, 1995) easily occurs to attack these microorganisms or to facilitate the formation of aseptic subcorneal pustules. We believe that the present findings lead to a better understanding of the defense mechanism against microorganisms and the pathogenesis of several sterile pustular dermatoses.

We thank Ms Yumiko Yoshino for skillful technical assistance. This study was supported in part by Shiseido Foundation for Skin Aging Research and by Grant-in-Aid Science Research, Nos. 06454313, 07807079, and 08457234 from the Ministry of Education, Science, and Culture, Japan.

REFERENCES

Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M, Fukami Y: Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem* 262:5592–5595, 1987

Alper CA, Johnson AM, Birtch AG, Moor FD: Human C3: evidence for the liver as the primary site of synthesis. *Science* 163:286–288, 1969

Andoh A, Fujiyama Y, Bamba T, Hosoda S: Differential cytokine regulation of complement C3, C4, and factor B synthesis in human intestinal epithelial cell line, Caco-2. *J Immunol* 151:4239–4247, 1993

Basset-Seguin N, Caughman SW, Yancey KB: A-431 cells and human keratinocytes synthesize and secrete the third component of complement. *J Invest Dermatol* 95:621–625, 1990

Bigler CF, Norris DA, Weston WL, Arend WP: Interleukin-1 receptor antagonist production by human keratinocytes. *J Invest Dermatol* 98:38–44, 1992

Botto M, Lissandrini D, Sorio C, Walport MJ: Biosynthesis and secretion of complement component (C3) by activated human polymorphonuclear leukocytes. *J Immunol* 149:1348–1355, 1992

Brenner DA, O'Hara M, Angel P, Chojkier M, Karin M: Prolonged activation of jun and collagenase genes by tumour necrosis factor-α. *Nature* 337:661–663, 1989

Brooimans RA, Stegmann AP, Van Dorp WT, Van Der Ark AA, Van Der Wounde FJ, Van Es LA, Daha MR: Interleukin 2 mediates stimulation of complement C3 biosynthesis in human proximal tubular epithelial cells. *J Clin Invest* 88:379–384, 1991

Brooimans RA, Van Der Ark AA, Buurman WA, Van Es LA, Daha MR: Differential regulation of complement factor H and C3 production in human umbilical vein endothelial cells by IFNγ and IL-1. *J Immunol* 144:3835–3840, 1990

Dvir A, Milner Y, Chomsky O, Gilon C, Gazit A, Levitzki A: The inhibition of EGF-dependent proliferation of keratinocytes by tyrphostin tyrosine kinase blockers. *J Cell Biol* 113:857–865, 1991

Filvaroff E, Calautti E, Reiss M, Dotto GP: Functional evidence for an extracellular calcium receptor mechanism triggering tyrosine kinase activation associated with mouse keratinocyte differentiation. *J Biol Chem* 269:21735–21740, 1994

Gazit A, Yaish P, Gilon C, Levitzki A: Tyrphostins I: synthesis and biological activity of protein tyrosine kinase inhibitors. *J Med Chem* 32:2344–2352, 1989

George MD, Vollberg TM, Floyd EER, Stein JP, Jetten AM: Regulation of transglutaminase type II by transforming growth factor-β1 in normal and transformed human epidermal keratinocytes. *J Biol Chem* 265:11098–11104, 1990

Hamilton AO, Jones L, Morrison L, Whaley K: Modulation of monocyte complement synthesis by interferons. *Biochem J* 242:809–815, 1987

Hayakawa M, Ishida N, Takeuchi K, Shibamoto S, Hori S, Oku N, Ito F, Tsujimoto M: Arachidonic acid-selective cytosolic phospholipase A2 is crucial in the cytotoxic action of tumor necrosis factor. *J Biol Chem* 268:11290–11295, 1993

Hunter T, Cooper JA: Protein tyrosine kinases. *Annu Rev Biochem* 54:897–930, 1985

Katz Y, Revel M, Strunk RC: Interleukin 6 stimulates synthesis of complement proteins factor B and C3 in human skin fibroblasts. *Eur J Immunol* 19:983–988, 1989

Katz Y, Strunk RC: IL-1 and tumor necrosis factor: similarities and differences in stimulation of expression of alternative pathway of complement and IFN-β_2/IL-6 genes in human fibroblasts. *J Immunol* 142:3862–3867, 1989

Kock A, Schwarz T, Kirnbauer R, Urbanski A, Perry P, Ansel JC, Lugar TA: Human keratinocytes are a source for tumor necrosis factor α: Evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. *J Exp Med* 172: 1609–1614, 1990

Kulics J, Colten HR, Perlmutter DH: Counterregulatory effects of interferon-γ and endotoxin on expression of the human C4 genes. *J Clin Invest* 85:943–949, 1990

Kupper TS: The activated keratinocytes: A model for inducible cytokine production by non-bone marrow-derived cells in cutaneous inflammatory and immune responses. *J Invest Dermatol* 94:146S–150S, 1990

Lappin DF, Guc D, Hill A, McShane T, Whaley K: Effect of interferon-γ on complement gene expression in different cell types. *Biochem J* 281:437–442, 1992

Lenardo MJ, Baltimore D: NF-κB: a pleiotropic mediator of inducible and tissue-specific gene control. *Cell* 58:227–229, 1989

Matsui MS, Chew SL, DeLeo VA: Protein kinase C in normal human epidermal keratinocytes during proliferation and calcium-induced differentiation. *J Invest Dermatol* 99:565–571, 1992

Perlmutter DH, Dinarello CA, Punsal PI, Colten HR: Cachectin/tumor necrosis factor regulates hepatic acute-phase gene expression. *J Clin Invest* 78:1349–1354, 1986a

Perlmutter DH, Goldberger G, Dinarello CA, Mizel SB, Colten HR: Regulation of class III major histocompatibility complex gene products by interleukin-1. *Science* 232:850–852, 1986b

Ramadori G, Van Damme J, Rieder H, Meyer Zum Buschenfelde KH: Interleukin 6, the third mediator of acute-phase reaction, modulates hepatic protein synthesis in human and mouse: Comparison with interleukin 1β and tumor necrosis factor-α. *Eur J Immunol* 18:1259–1264, 1988

Reiners Jr JJ, Slaga TJ: Effects of tumor promoters on the rate and commitment to terminal differentiation of subpopulations of murine keratinocytes. *Cell* 32:247–255, 1983

Sackstein R, Colten HR: Molecular regulation of MHC class III (C4 and factor B) gene expression in mouse peritoneal macrophages. *J Immunol* 133:1618–1626, 1984

Saunders NA, Jetten AM: Control of growth regulatory and differentiation-specific genes in human epidermal keratinocytes by interferon γ. Antagonism by retinoic acid and transforming growth factor β1. *J Biol Chem* 269:2016–2022, 1994

Strunk RC, Eidlen DM, Mason RJ: Pulmonary elveolar type II epithelial cells synthesize and secrete proteins of the classical and alternative complement pathways. *J Clin Invest* 81:1419–1426, 1988

Terui T, Kato T, Tagami H: Stratum corneum activation of complement through the antibody-independent alternative pathway. *J Invest Dermatol* 92:593–597, 1989

Terui T, Zhen Y-X, Kato T, Tagami H: Mechanism of human polymorphonuclear leukocyte adhesion to serum-treated corneocytes. *J Invest Dermatol* 104:297–301, 1995

Uehara Y, Fukazawa H, Murakami Y, Mizuno S: Irreversible inhibition of v-src tyrosine kinase activity by herbimycin A and its abrogation by surfhydryl compounds. *Biochem Biophys Res Commun* 163:803–809, 1989

Westwick JK, Weitzel C, Minden A, Karin M, Brenner DA: Tumor necrosis factor α stimulates AP-1 activity through prolonged activation of the c-Jun kinase. *J Biol Chem* 269:26396–26401, 1994

Yancey KB, Overholser O, Domloge-Hultsch N, Li L-J, Caughman SW, Bisalbutra P: Human keratinocytes and A-431 cells synthesize and secrete factor B, the major zymosan protease of the alternative complement pathway. *J Invest Dermatol* 98:379–383, 1992

Yang Z, Costanzo M, Golde DW, Kolesnick RN: Tumor necrosis factor activation of the sphingomyelin pathway signals nuclear factor kB translocation in intact HL-60 cells. *J Biol Chem* 268:20520–20523, 1993

Yuspa SH, Ben T, Hennings H, Lichit U: Phorbol ester tumor promoters induce epidermal transglutaminase activity. *Biochem Biophys Res Commun* 97:700–708, 1982

Zhao Y, Uyttendaele H, Krueger JG, Sudol M, Hanafusa H: Inactivation of c-Yes tyrosine kinase by elevation of intracellular calcium levels. *Mol Cell Biol* 13:7607–7514, 1993